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Plasma Cotinine: Stability in Smokers and Validation of Self-Reported Smoke Exposure in Nonsmokers

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The stability of plasma cotinine was studied in smokers and the validity of a brief questionnaire on environmental tobacco smoke (ETS) exposure was assessed in nonsmokers. The correlation between two cotinine levels measured 14 weeks apart was 0.81 in smokers ($n = 148$). A single measurement will classify 95% of all subjects within 30% of their habitual plasma cotinine level. Plasma cotinine was higher in ETS-exposed nonsmokers ($n = 50$) than in ETS-unexposed nonsmokers ($n = 55$; 1.6 ng/ml vs 0.6 ng/ml; $P < 0.0001$), but there was a substantial part of intersubject overlap. The sensitivity and specificity with respect to self-reported smoke exposure were 56 and 89%, respectively. This study shows that a single plasma cotinine determination gives a good impression of cotinine levels. It is dubious if a brief questionnaire about hours of passive smoke exposure in nonsmokers is valid to distinguish ETS-exposed from ETS-unexposed nonsmokers.

INTRODUCTION

Cigarette smoking is important in the etiology of many diseases, e.g., lung cancer and cardiovascular disease (Multiple Risk Factor Intervention Trial Research Group, 1982). As a consequence, correct identification of smoking status and tobacco smoke exposure is of paramount importance in many etiologic studies.

Self-reports of smoking behavior may not always be reliable. It is suggested that the increasing social unacceptability of smoking could result in significant underreporting (Jarvis *et al.*, 1987; Sillett *et al.*, 1978; Pojer *et al.*, 1984). A number of biochemical markers, like nicotine, cotinine, thiocyanate, and carbon monoxide, have been used to validate reported smoking behavior and passive smoke exposure.

Cotinine, the major metabolite of nicotine, seems the most valid indicator of smoke absorption, either actively or passively, during the past few days (Jarvis *et al.*, 1987; Hill *et al.*, 1983; Wald *et al.*, 1984). The sensitivity and specificity of the cotinine assay for distinguishing smokers from nonsmokers are high (88–100%) (Jarvis *et al.*, 1987; Van Vunakis *et al.*, 1989), although there is a relatively high degree of intersubject variability of cotinine levels among subjects claiming to smoke the same number of cigarettes per day (Hill *et al.*, 1983; Matsukura *et al.*,

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1984; Wall *et al.*, 1988). This mainly reflects individual differences in patterns of puffing and inhalation (Hill *et al.*, 1983; Wall *et al.*, 1988; Langone *et al.*, 1973).

At this moment it is not known whether a single cotinine measurement is a valid indicator of long-term smoking behavior. In the present study therefore, we calculated stability of plasma cotinine levels measured at the beginning and at the end of a 14-week period in smokers in whom smoking behavior was not changed. From these data we calculated how many measurements of plasma cotinine are needed for the correct classification of subjects on their habitual cotinine level. In addition, we report cotinine levels in nonsmokers selected for the extent of their exposure to environmental tobacco smoke. Furthermore, we set out to assess the validity of a questionnaire on self-reported passive smoke exposure.

SUBJECTS AND METHODS

Study Design

We studied healthy male volunteers who participated in an intervention study on the effect of β-carotene supplementation on genotoxicity indices in smokers (Van Poppel *et al.*, 1992). These subjects were enlisted by an advertisement in an in-house company newsletter of three administrative companies. Volunteers were asked to fill in a questionnaire on smoking habits and passive exposure to tobacco smoke (number of smokers in the home environment, number of smoking colleagues at the work place, and estimated hours of exposure to tobacco smoke weekly). Table 1 shows the characteristics of the subjects.

Smoking subjects were selected ($n = 163$) when they smoked at least 15 cigarettes a day for over 2 years. Nonsmokers were selected from the lower and upper tail of the distribution of self-reported hours of passive exposure to environmental tobacco smoke (ETS). These participants had not smoked actively in the last 5 years.

The ETS-unexposed nonsmokers ($n = 55$) had no smokers in the home environment. They reported a maximum of 10 hr a week passive exposure to environmental tobacco smoke, with the exception of 4 with between 10 and 20 hr a week. Only 8 had smoking colleagues at work, and these reported less than 10 hr of total passive exposure a week. The ETS-exposed nonsmokers ($n = 50$) all had one or more smokers in the home environment. Eight reported passive exposure of environmental tobacco smoke between 20 and 40 hr a week, and all others reported at least 40 hr of exposure a week. Except for 10 subjects, all had smoking colleagues at the work place.

Two blood samples were collected from smokers before and after a study period of 14 weeks. During this 14 weeks the subjects were randomly assigned to either a β-carotene (20 mg/day) or placebo treatment (Van Poppel *et al.*, 1992). The subjects were asked to maintain their habitual smoking behavior. One single blood sample was collected from ETS-exposed and ETS-unexposed nonsmokers at the beginning of the study period.

During the trial, one smoking subject lost weight (±10 kg) and one reported having changed his smoking behavior from three to one packet of cigarette tobacco a week. This subject is excluded for the analyses in this study, although his

plasma cotinine concentration hardly changed during the study period (349.0 ng/ml vs 313.2 ng/ml, respectively). A total of 14 smokers withdrew participation during the 14-week trial. This left 148 smokers and 55 ETS-unexposed and 50 ETS-exposed nonsmokers for data analyses in this study.

Analytical Methods

Nonfasting blood was collected by venapuncture between 8.30 and 14.00 hr. All blood samples were stored in the dark at 0–4°C. After 20–23 hr, plasma was separated and stored at −80°C. Plasma cotinine levels were determined by gas chromatography with a detection limit of 0.1 ng/ml (Feyerabend and Russell, 1990).

For each smoker, plasma cotinine levels of the initial and final collection (respectively cotinine 1 and cotinine 2) were determined simultaneously, to eliminate a possible between-run variation. Accuracy was checked by the analysis of two Na–EDTA plasma pools. In every run, random blinded split samples (7 for a nonsmoker and 17 for a smoker) were included to check the accuracy of the methodology. The overall coefficient of variation over all runs was 3%.

Data Analysis

To evaluate if the β-carotene– and placebo-supplemented smokers could be analyzed as one group, differences in cotinine levels between these groups, before as well as after the study period, were tested for significance with the Student's *t* test. The data of the ETS-unexposed and ETS-exposed nonsmokers did not appear to be normally distributed; therefore, the Mann–Whitney rank sum test was used to test the significance of differences in initial cotinine levels between smokers, ETS-unexposed, and ETS-exposed nonsmokers. To validate the questionnaire on self-reported smoke exposure in nonsmokers, the sensitivity (percentage of ETS-exposed nonsmokers correctly classified) and specificity (percentage of ETS-unexposed nonsmokers correctly classified) were calculated by selecting the value which, in relation to self-reported passive smoke exposure, misclassified the fewest subjects.

The relationship between cotinine 1 and cotinine 2 just as the relationship between the mean cotinine level and the reported cigarette consumption for smokers was examined by calculating linear regression equations. For ETS-exposed and ETS-unexposed nonsmokers, the relationship of cotinine 1 and hours of passive smoke exposure was also examined by calculating linear regression equations. Correlations were measured by Pearson's product moment correlation coefficient. The between-person variance in mean cotinine level was calculated using analysis of variance (one-way).

The number of measurements (k) needed to estimate the individual cotinine level within a certain percentage (D) of a person's habitual cotinine level with a probability of 95% was calculated using the equation

$$k = \left(1.96 * \frac{CV}{D}\right)^2,$$

where CV is the within-person coefficient of variation (Knuiman *et al.*, 1988).

Data analyses were performed with the use of the Statistical Analysis System software package version 6.06 (SAS, 1990).

RESULTS

No significant difference was found in plasma cotinine level between the placebo and β-carotene group, before as well as after the study period. Supplementation of β-carotene did not affect the cotinine level, so the pooled data could be used.

In Table 1 some characteristics of smokers, ETS-unexposed, and ETS-exposed nonsmokers in this study are presented. These groups are comparable for most of the characteristics. A small difference was observed in Quetelet index between ETS-exposed and ETS-unexposed nonsmokers, with ETS-exposed nonsmokers appearing to be slightly heavier. As expected, cotinine 1 was significantly higher in the smokers than in the total nonsmokers group ($P < 0.0001$, Mann–Whitney test).

The contrast in self-reported exposure to environmental tobacco smoke was considerable: an average of 4.5 hr a week for the ETS-unexposed group compared to 72.8 hr a week for ETS-exposed nonsmokers. This contrast was reflected in differences in plasma cotinine levels as presented in Fig. 1. For the ETS-unexposed nonsmokers the mean plasma cotinine concentration was 0.6 ng/ml

TABLE 1
CHARACTERISTICS OF THE SAMPLE POPULATION

Variable	Smokers ($n = 148$)	ETS[a]-exposed nonsmokers ($n = 50$)	ETS[a]-unexposed nonsmokers ($n = 55$)
Age (year)	39.3 (9.8)	37.5 (10.2)	39.0 (10.3)
Weight (kg)	78.9 (11.3)	81.2 (10.2)	78.2 (8.8)
Length (cm)	179.6 (7.5)	180.2 (7.1)	180.4 (5.7)
QI (kg/m^3)	24.4 (2.9)	25.0 (2.7)	24.0 (2.6)
Smoke duration (year)	21.1 (10.1)	—	—
Reported consumption of tobacco products			
Cigarettes/day	21.2 (6.5)	—	—
Cigars/day	0.04 (0.4)	—	—
Pipes/day	0	—	—
Passive smoking (hr/week)			
At work	—	28.7 (14.2)	2.5 (3.1)
At home	—	35.3 (22.1)	0.1 (0.7)
Elsewhere	—	9.0 (9.4)	0.9 (2.1)
Total	—	72.8 (33.1)	4.5 (3.6)
Cotinine 1[b] (ng/ml)	327.1 (116.2)	1.6 (1.5)	0.6 (0.5)
Cotinine 2[c] (ng/ml)	316.9 (119.1)	—	—

Note. Data shown are means (SD). Numbers for each variable may differ from the bases because of missing data.

[a] ETS, environmental tobacco smoke.

[b] Plasma cotinine level before the study period.

[c] Plasma cotinine level after the study period (14 weeks).

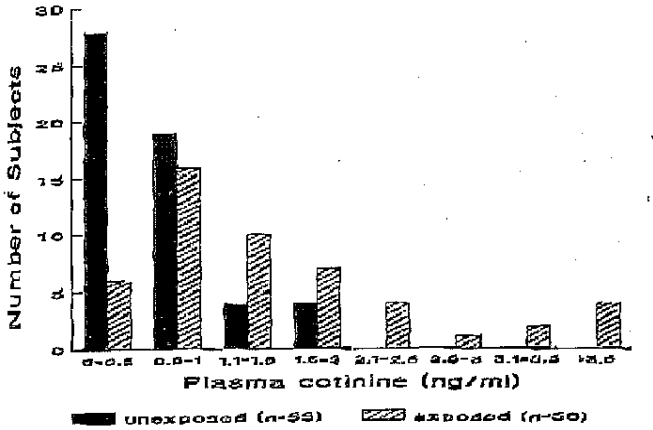

FIG. 1. Plasma cotinine levels in ETS-unexposed and ETS-exposed nonsmokers.

(range: 0.0–1.9). For the ETS-exposed nonsmokers the mean was 1.6 ng/ml (range: 0.4–9.0); ($P < 0.0001$, Mann–Whitney test). As a consequence, there was a clear correlation between hours of passive smoke exposure and cotinine 1 in the total nonsmoker group ($\beta = 0.01$ (ng/ml per hour smoke exposure), $P < 0.0001$, $r = 0.42$). However, within the ETS-exposed and ETS-unexposed group this relation was no longer present (ETS-exposed nonsmokers: $\beta = 0.006$, $P > 0.35$, $r = 0.14$; ETS-unexposed nonsmokers: $\beta = 0.03$, $P > 0.12$, $r = 0.24$). The cut-off value which misclassified the fewest subjects in relation to self-reported passive smoke exposure was 1.1 ng cotinine per milliliter of plasma. The specificity of self-reported passive smoke exposure was rather high: 89%. The sensitivity however was 56%.

The within-person coefficient of variation in cotinine levels for smokers was 16.1%, and the between-person coefficient of variation in mean cotinine level was 22.3%. In univariate regression analysis for smokers, cotinine 1 was positively associated with cotinine 2 (see Fig. 2) with a high explained proportion of variance ($\beta = 0.83$ (ng/ml), $P < 0.0001$, $r = 0.81$). The standard error of the regression line was 0.05 ng/ml. The reported cigarette consumption was related to the mean cotinine level ($\beta = 7.44$, $P < 0.0001$, $r = 0.44$). However, the explained proportion of variance was low. Regression analyses were done including age, years of smoking, and Quetelet index separately with cotinine 1 as a predictor of the cotinine 2 level. For all of these variables, the regression coefficient of cotinine 1 and the amount of variation explained by the model did not change, so these variables are not considered confounders. The same result was found for age and Quetelet index with ETS exposure as predictor of the cotinine 1 level for nonsmokers.

Figure 3 gives the number of measurements needed to estimate the individual cotinine level within different percentages of a person's habitual cotinine level (95% confidence interval). A single measurement will classify 95% of all subjects

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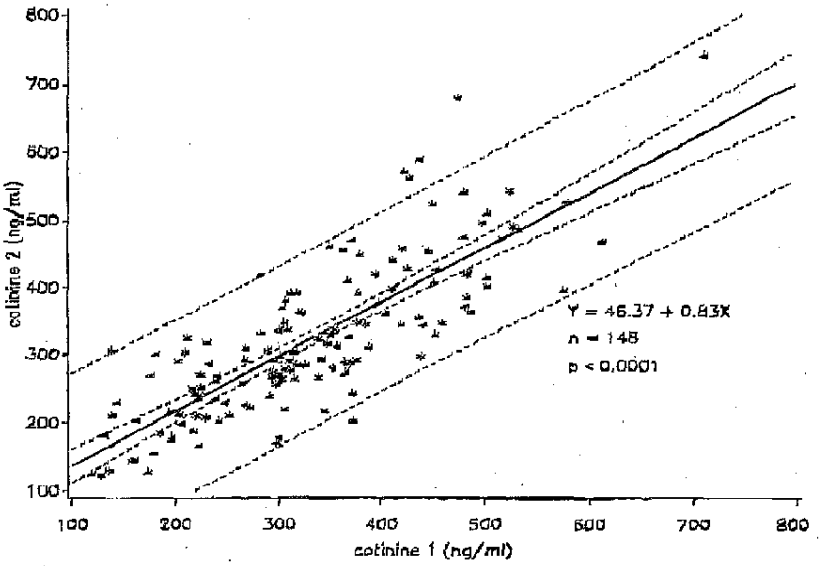

FIG. 2. Regression line of the initial (cotinine 1, ng/ml) and final (cotinine 2, ng/ml) cotinine measurement with 95% confidence limits for the mean and individual predicted values.

within 30% of their "true" plasma cotinine level. For classification within 10% of their habitual level, nine measurements are needed.

DISCUSSION

In this study, a correlation in smokers was found between the initial (cotinine 1) and final (cotinine 2) plasma cotinine level within a study period of 14 weeks. This indicates that a single plasma cotinine determination gives a good impression of cotinine levels over a longer term in people having stable smoking habits. Furthermore, this study demonstrated that the sensitivity (percentage of ETS-exposed nonsmokers correctly classified) and specificity (percentage of ETS-unexposed nonsmokers correctly classified) for self-reported passive smoke exposure were 56 and 89%, respectively.

The results clearly show that the cotinine 1 level in smokers is significantly higher than that in nonsmokers. This finding is in agreement with previous findings of Muranaka *et al.* (1988), Pojer *et al.* (1984), and Jarvis *et al.* (1987). Also, a higher cotinine level is found in ETS-exposed nonsmokers than in ETS-unexposed nonsmokers. This is in agreement with findings of Matsukura *et al.* (1984), Wald *et al.* (1984), and Haley *et al.* (1989). Cotinine in body fluids thus seems a useful marker of exposure to other people's tobacco smoke. However, despite a great contrast between the nonsmoker groups, created by selection of the subjects on self-reported exposure, a substantial overlap in cotinine levels between ETS-unexposed and ETS-exposed nonsmokers was observed, resulting in a low sensitivity of 56% (see Fig. 1). This intersubject overlap was also found

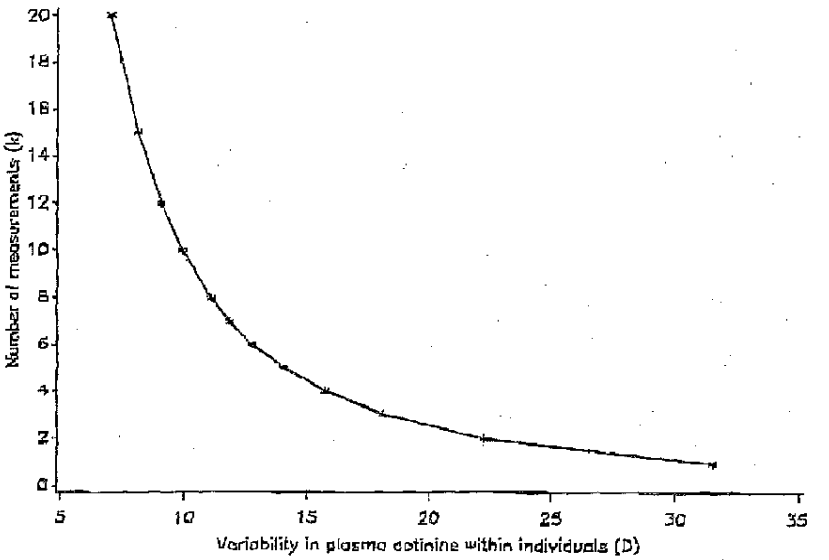

FIG. 3. Relation between the number of measurements (k) needed to estimate the individual cotinine level within different percentages (D) of a person's habitual cotinine level (95% confidence interval).

by Wall *et al.* (1988) and indicates that a number of subjects will be misclassified according to self-reported passive smoke exposure. It is doubtful if a brief questionnaire about hours of passive smoke exposure is valid to distinguish ETS-exposed from ETS-unexposed nonsmokers. This could be explained by the fact that the degree of passive smoke exposure is not only a function of time an individual spends in each setting but also the concentration of tobacco-related air pollutants in that environment (Haley *et al.*, 1989) and perhaps over or underreporting of passive smoke exposure (Wall *et al.*, 1988) can play an important role.

We found in this study a strong correlation between cotinine 1 and cotinine 2 in people having stable smoking habits. It has been assumed (Hill *et al.*, 1983; Zeidenberg *et al.*, 1977) that cotinine levels remain fairly constant in individuals who smoke according to a consistent pattern. However, this assumption is based on a study of Langone *et al.* (1973) in which sera samples from only two smokers were analyzed for cotinine with a very short interim period (3 days before and 8 days after they stopped smoking). This makes our study the first report on this matter for a greater study group over a longer study period. The regression coefficient of the relation between cotinine 1 and cotinine 2 in smokers having stable smoking habits is somewhat below the theoretically expected value of 1.0. Probably, this is due to regression to the mean (see Fig. 2). The correlation of 0.81 is rather high for an epidemiologic study, since the study was not carried out in a laboratory setting and was thus more prone to random errors. Rigid by variations in time period between blood sampling and plasma separation, processing of the plasma samples or the amount of blood drawn into a tube is often not feasible. The

within-person variation could also be explained by changing style of smoking in time, like differences in inhalation and the number of puffs. Analytical error can only be expected to have a minor contribution to the variation over time, given the coefficient of variation of only 3%.

The correlation of 0.44 in this study found between the reported cigarette consumption and the mean cotinine level is comparable to previous reports. Hill *et al.* (1983) reported a correlation of 0.45 among men, Pojer *et al.* (1984) reported 0.34 among men and women, and Pierce *et al.* (1987) reported a correlation with saliva cotinine of 0.33 among men and women. These rather low correlations could reflect inaccurate reporting of the number of cigarettes smoked, variability in strength of cigarettes, and difference between subjects in metabolism, depth, and frequency of inhalation, unsmoked butt length, and obstruction of filter ventilation (Pojer *et al.*, 1984; Langone *et al.*, 1973). Our study had no measures of these variables, so we cannot evaluate the effect of these factors.

We estimate a deviation around the habitual plasma cotinine level in individuals of 30% by one measurement and 18% by three measurements in subjects having stable smoking habits. This within-person variability is of the same magnitude as for plasma cholesterol, a frequently used biomarker. Thompson and Pocock (1990) observed 28% deviation with a single cholesterol measurement and 20 or 14% by the average of two or four measurements, respectively, whereas Scaccini *et al.* (1991) report lower values (10% for one and 6% for three measurements). However, the blood samples for cholesterol measurements in the study of Scaccini *et al.* (1991) were drawn in a short study period (four mornings during 10 days). Our study period was 14 weeks so the chance that the within-person variation will rise is much greater.

In conclusion, this is the first study that indicates that a single plasma cotinine determination gives a good impression of cotinine levels over a longer term in people smoking more than 15 cigarettes a day and having stable smoking habits. Plasma cotinine seems to be a valid indicator of smoke behavior in these people. It should be realized, however, that the regression equation in Fig. 2 bears upon heavy smokers and cannot readily be extrapolated to cotinine levels below 100 ng/ml. It remains to be established whether the results are also applicable to light smokers and to people having irregular smoking habits. Furthermore, it is doubtful if a brief questionnaire about hours of passive smoke exposure in nonsmokers is valid to distinguish ETS-exposed from ETS-unexposed nonsmokers. Further studies with a more detailed questionnaire about intensity of passive smoke exposure are needed to evaluate the effectiveness of this variable.

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